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An effective family shuffling method using single-stranded DNA.

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Family shuffling, which is one of the most powerful techniques for in vitro protein evolution, always involves the problem of reassembling the gene fragments into parental gene sequences, because such a process prevents the formation of chimeric sequences. In order to improve the efficiency of hybrid formation in family shuffling, single-stranded DNAs (ssDNAs) were used as templates. The ssDNAs of two catechol 2,3-dioxygenase genes, nahH and xylE, were prepared, the xylE strand being complementary to the nahH strand. When these ssDNAs were digested by DNase I and reassembled, chimeric genes were obtained at a rate of 14%, which was much higher than the rate of less than 1% obtained by shuffling with double-stranded DNAs. Chimeric catechol 2,3-dioxygenases that were more thermally stable than the parental enzymes, XylE and NahH, were obtained by this ssDNA-based DNA shuffling.

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